

Fig. 1

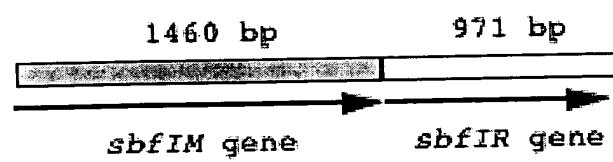


Figure 2-1

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GTGCATCCGATCGCCAGCACTGAAACTCGCCGCCAAGCTGCTCTCGGCCAAACTGGACCCC
1 -----+-----+-----+-----+-----+-----+-----+ 60
M H P I A S T E T R R Q A A L G K L D P
ACTACTCAAGCGGTGCTAGGGCAGTTCTTCACTCCCATGAAGGCCGCCACGCTGATGGCT
61 -----+-----+-----+-----+-----+-----+-----+ 120
T T Q A V L G Q F F T P M K A A T L M A
TCAATGCTTCGGGTCGATGATCTCCGCGGAACGGTGCGGGTGCTCGACCCAGGAGCTGGT
121 -----+-----+-----+-----+-----+-----+-----+ 180
S M L R V D D L R G T V R V L D P G A G
GTCGGGTCTCTGACCGCTGCCCTCGTCGATCGGCTGCATACTGAACGCCCCGACGTTGCG
181 -----+-----+-----+-----+-----+-----+-----+ 240
V G S L T A A L V D R L H T E R P D V A
GTCCACGTAGTTGCCGTGGAACCGACCCCTTTGTCGTGCCTTACCTGCGCGCCACCCTG
241 -----+-----+-----+-----+-----+-----+-----+ 300
V H V V A V E T D P F V V P Y L R A T L
GAGGAATGTCGGAACGCTTACGGCATCTCCTACGACCTAGTCGAGGGCGACTATTTGCTT
301 -----+-----+-----+-----+-----+-----+-----+ 360
E E C R N A Y G I S Y D L V E G D Y L L
AACCAAGGGGCCAAGCTGGATGGCCCGTTTCGATCTTGTAATTGCTAATCCTCCCTACGGA
361 -----+-----+-----+-----+-----+-----+-----+ 420
N Q G A K L D G P F D L V I A N P P Y G
AAGCTTGCTTCAGATTCGCTGGCGCGGCTTGCAACGACAGCGCGTGCCGTCGATGTACCG
421 -----+-----+-----+-----+-----+-----+-----+ 480
K L A S D S L A R L A T T A R A V D V P
AACGTTTACGTGGCCTTCTGGGTGCGAGCAGTCATTTGCTCAAAGAGCAGGGGCGGGGG
481 -----+-----+-----+-----+-----+-----+-----+ 540
N V Y V A F W V R A V I S L K E Q G R G
GTTTTTCATTGTTCTCGATCTTGGGCGAACGGGCCTTACTATCGTCAATTTCGCCATTGG
541 -----+-----+-----+-----+-----+-----+-----+ 600
V F I V P R S W A N G P Y Y R Q F R H W
CTGATGACCGCGTAAGTCTCGATATACTTCATGTGTTCGAAAGTAGAACCAAAGTATTT
601 -----+-----+-----+-----+-----+-----+-----+ 660
L M T A V S L D I L H V F E S R T K V F
GCGGACACGAAGGTAAAGCAAGAGAATGTCATCGTTGCTTTCAGTGTGAGGCCGCAAAGC
661 -----+-----+-----+-----+-----+-----+-----+ 720
A D T K V K Q E N V I V A F S V R P Q S
TCTAGTGTGGTCTTCTAGGTCGGTCGCACATGGAGAAGAGTCGATCGCAAGTTCTGTG
721 -----+-----+-----+-----+-----+-----+-----+ 780
S S V V L S R S V A H G E E S I A S S V
CCGTTTTCTGCGCTTGTTCATGATGAAGACGATGACAAAATCGTGCACCTTCGCGGAAAGC
781 -----+-----+-----+-----+-----+-----+-----+ 840
P F S A L V H D E D D D K I V H F A E S
GCATCGGTGCCCCGCGCGGAGGTTTACTCTCGCTGATCTCGGCATCGGTGTAAGTACG
841 -----+-----+-----+-----+-----+-----+-----+ 900
A S V P S A A R F T L A D L G I G V S T
GGAAAGGTTGTTGATTTTCGCAATCGTCAGTATTTGACCGATAACCTGGATGCTTCAGGC
901 -----+-----+-----+-----+-----+-----+-----+ 960
G K V V D F R N R Q Y L T D N L D A S G
GTTGTGCCCATGGTTTATCAGTCAAACATTCGATCTGGTAAAATTGATTGGCCTCAGGTG
961 -----+-----+-----+-----+-----+-----+-----+ 1020
V V P M V Y Q S N I R S G K I D W P Q V
GGTGCGAGGAAGCCTCAAGGATTTGTTGCGGTGCAAGATGTAGCACTACGTCAACTTCTC
1021 -----+-----+-----+-----+-----+-----+-----+ 1080
G A R K P Q G F V A V E D V A L R Q L L

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Figure 2-2

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1081  CCGCAAGGGTCGTATGTTGTTGTGAAACGGCAAACGGCGAAAGAGGACCGTCGTCGTGTC 1140
      -----+-----+-----+-----+-----+
      P Q G S Y V V V V K R Q T A K E D R R R V
1141  ATCGCTGCGGTCTGGGACGGGGCCAGCAGGGTTGCGCTCGACAATAAAACGAAC TATTG
      -----+-----+-----+-----+-----+
1201  I A A V W D G A S R V A L D N K T N Y L
      CATGAATCTCAACGACCGCTTGAGAAAAATGTGGCCCGGGCCTCATGCTTTGGTTGAAC
1201  -----+-----+-----+-----+-----+ 1260
      H E S Q R P L E K N V A R G L M L W L N
      TCGACTGTGTTGGATCAGTATTTCCGAGCCTTTTCCGGGCATACCCAGGTGAACGCTGGC
1261  -----+-----+-----+-----+-----+ 1320
      S T V L D Q Y F R A F S G H T Q V N A G
      GATCTACGCCGGCTTCCGTTCTCTGTGCGGAGGACCTAATTCTTCTCGCTAAGGTCGTT
1321  -----+-----+-----+-----+-----+ 1380
      D L R R L P F L C R E D L I L L A K V V
      CCCGATGGCCTGCCTGATCAGGAGACGTTGGATGCCGTGGTGGCCAGACTCTTCTGTGAG
1381  -----+-----+-----+-----+-----+ 1440
      P D G L P D Q E T L D A V V A R L F C E
      ATTCCGGAATCTGCCTCGTGA
1441  -----+-----+-- 1461
      I P E S A S *
```

Figure 3

```

GTGAACAGCAGTGACGGCATCGACGGAACGGTAGCGAGCATCGATACTGCGCGGGCGCTG
1  -----+-----+-----+-----+-----+-----+-----+ 60
M N S S D G I D G T V A S I D T A R A L
CTAAAGCGTTTTGGGTTTGACGCGCAACGATATAACGTCCGTAGCGCTGTGACATTGCTC
61  -----+-----+-----+-----+-----+-----+-----+ 120
L K R F G F D A Q R Y N V R S A V T L L
GCGCTTGCCGGTTTGAAGCCGGGAGATCGCTGGGTTGACTCGACCACTCCACGCCTTGGC
121  -----+-----+-----+-----+-----+-----+-----+ 180
A L A G L K P G D R W V D S T T P R L G
GTTTCAAGATCATGGACTGGTCCGGCGAGCATTGGGCCAAGCCGTACGCCACCGGAAGT
181  -----+-----+-----+-----+-----+-----+-----+ 240
V Q K I M D W S G E H W A K P Y A T G S
CGAGAAGATTTCCGTAAGAAGACGCTTCGGCAGTGGGTTGATAATGGCTTCGCCGTACTT
241  -----+-----+-----+-----+-----+-----+-----+ 300
R E D F R K K T L R Q W V D N G F A V L
AATGCGGACAATTTAAACATCGCCACGAACCTCGCAGCTCAACGAGTACTGCTTGTCTGAC
301  -----+-----+-----+-----+-----+-----+-----+ 360
N A D N L N I A T N S Q L N E Y C L S D
GAAGCATTACAGGCGCTAAGGGCATATGGAACGGAAGGCTTCGAGGAATCTCTTGTAGTC
361  -----+-----+-----+-----+-----+-----+-----+ 420
E A L Q A L R A Y G T E G F E E S L V V
TTTCTTGATGAAGCATCGAAGGCGGTTAAAGCGCGAGCGGAAGCTCTCCAGGCTGCGATG
421  -----+-----+-----+-----+-----+-----+-----+ 480
F L D E A S K A V K A R A E A L Q A A M
ATCTCTGTCGATCTCCCTGGTGGCGAGGAATTTCTGCTCTCGCCTGCCGGGCAGAATCCA
481  -----+-----+-----+-----+-----+-----+-----+ 540
I S V D L P G G E E F L L S P A G Q N P
TTGCTGAAGAAGATGGTTCGAAGAGTTTGTGCCGCGATTTGCACCTCGCTCGACGGTGCTC
541  -----+-----+-----+-----+-----+-----+-----+ 600
L L K K M V E E F V P R F A P R S T V L
TACCTCGGGGATACTCGTGAAAGCATTCCCTATTTCGAACGAGAGATCTTTGAAGAGGTG
601  -----+-----+-----+-----+-----+-----+-----+ 660
Y L G D T R G K H S L F E R E I F E E V
CTCGGCCTGACTTTTCGACCCCATGGTTCGAATGCCGGACCTTATTCTCCATGACGAAGTT
661  -----+-----+-----+-----+-----+-----+-----+ 720
L G L T F D P H G R M P D L I L H D E V
CGTGGGTGGCTTTTCCCTTATGGAGGCCGTGAAAAGTAAAGGTCCGTTTGATGAGGAGCGG
721  -----+-----+-----+-----+-----+-----+-----+ 780
R G W L F L M E A V K S K G P F D E E R
CATCGCAGCCTGCAAGAGCTATTTCGTTACACCTTCAGCGGGTCTAATTTTGTAAACTGC
781  -----+-----+-----+-----+-----+-----+-----+ 840
H R S L Q E L F V T P S A G L I F V N C
TTTGAAAATCGTGAGTCGATGCGTCAGTGGCTCCCTGAGCTGGCTTGGGAACTGAGGCG
841  -----+-----+-----+-----+-----+-----+-----+ 900
F E N R E S M R Q W L P E L A W E T E A
TGGGTAGCGGAAGATCCAGACCATCTGATTACCTTAACGGGTCTAGATTTCTTGGGCCG
901  -----+-----+-----+-----+-----+-----+-----+ 960
W V A E D P D H L I H L N G S R F L G P
TACGAACGTTAG
961  -----+-----+-----+-----+-----+-----+-----+ 972
Y E R *

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Fig. 4 pstIM-pACYC184

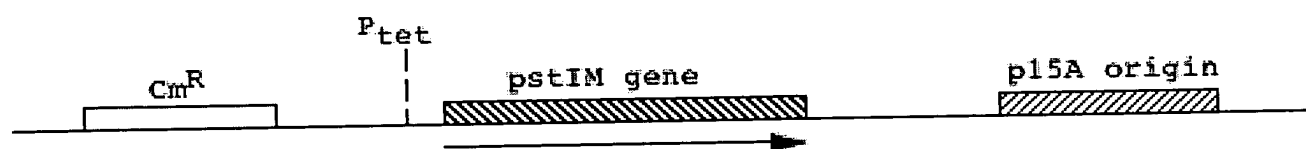


Fig. 5 sbfIM-pACYC184

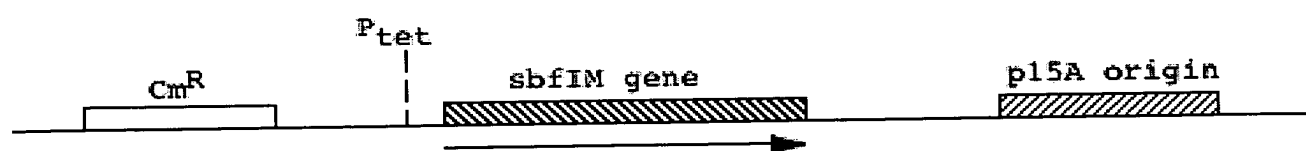
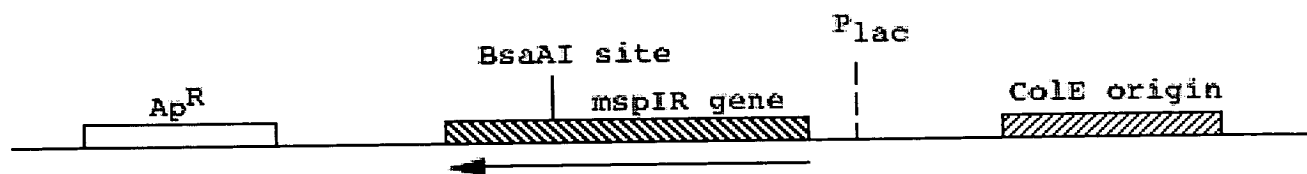
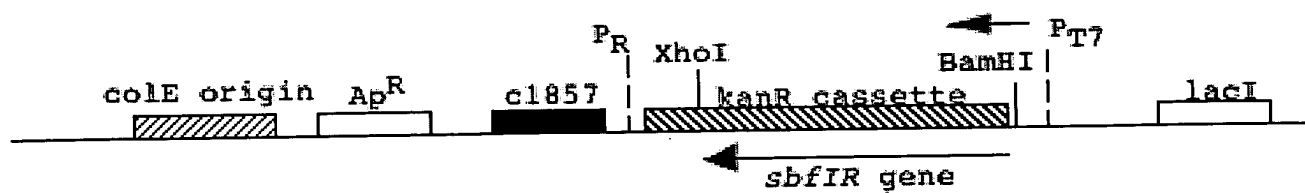


Fig. 6 pCAB16



Note: sbfIR or sbfIM cloned at BsaAI site

Fig. 7 sbfIR-pLT7K

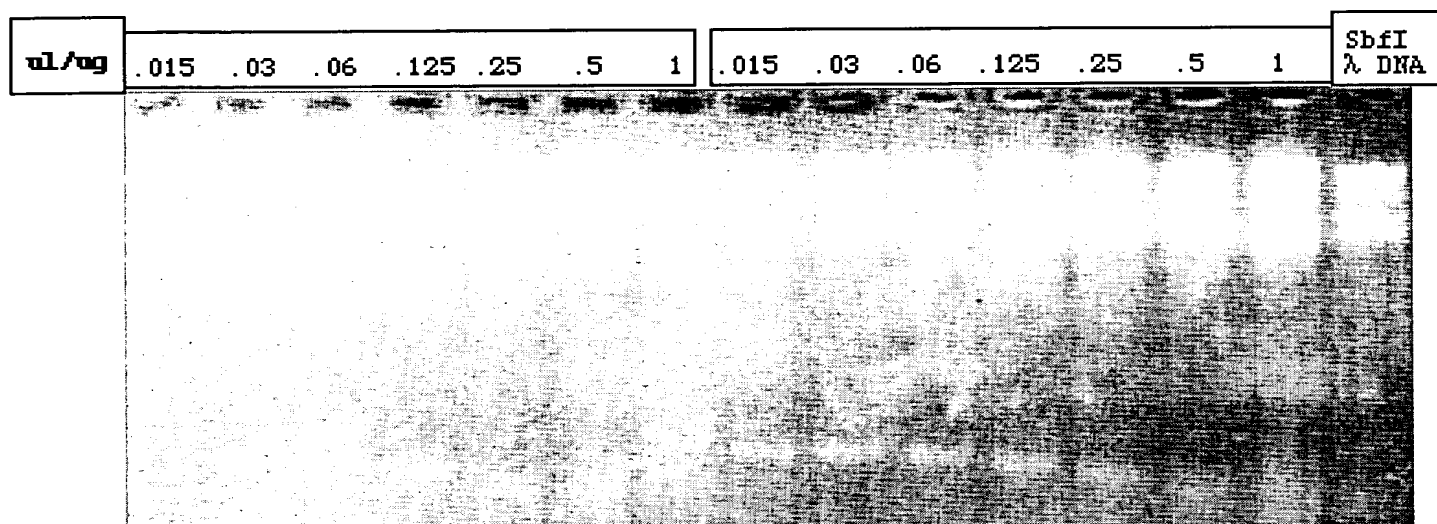


Note: sbfIR cloned at BamHI to XhoI site

Fig 8

Final Overexpression of SbfI

Endonuclease



NEB#1500, ER2848 [pACYC184-SbfIM #7, pLT7K-SbfIR #12]